# Proceedings of the conference 'New Frontiers in Oral Immunological Diseases', Lillehammer, Norway, 2001. Part 3

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## Editorial

## Immunological tolerance: the good, the bad, and the ugly

The following articles constitute the third and final part of the proceedings of the international conference 'New Frontiers in Oral Immunological Diseases', which was held in Lillehammer, Norway, 23–27 February 2001. The first and second sets of articles were published in the two previous issues of Acta Odontologica Scandinavica (1–20). The aim of the conference was to introduce new perspectives in oral immunological diseases through integration with non-oral fields. The present reviews cover the issues of mucosal immunity and adverse reactions after exposure to foreign environmental materials and biomaterials. Recent guidelines for biological evaluation of biomaterials and medical devices in the European Union are given in an addendum.

*Immunological systemic and oral tolerance—the 'good' side*

The acquired systemic and mucosal immune systems have the capability to recognize a great variety of antigens. Fortunately, however, the range of proteins that induce a response is limited by the phenomenon of tolerance. The main antigens that the host must tolerate are self-antigens, dietary and non-damaging environmental antigens, and antigens from the normal microflora on the skin and the mucosal surfaces. Various mechanisms contribute to central and peripheral tolerance, including clonal deletion, clonal inactivation (in the absence of co-stimulation), lack of a helper T-cell response, suppression by T cells, and, probably, other immunological processes. Mucosal tolerance has an additional important function: to suppress the generation of systemic immunity (in blood and tissue) against antigens in the mucosal tract ('oral tolerance'). This oral tolerance is protective because an excessive systemic response could lead to mucosal inflammation accompanied by tissue damage (5, 11, 17, 18). All these aspects of tolerance are essential for the health of the host.

*Immunological oral tolerance—the 'bad' side*

Acquired mucosal immunity is regulated differently from that of its systemic counterpart in blood and tissues. Mucosal immunity is triggered at various inductive sites in the body, such as the tonsils and Peyer patches. Activated cells are then distributed through the bloodstream to most of the body's mucosal effector sites, including the salivary glands and the intestinal lamina propria. This aggregate of sites and cells and the traffic and the signaling between them constitute what is known as the common mucosal immune system (CMIS). The mucosa is exposed to a wealth of antigens derived from dietary sources and the normal bacterial flora, so the CMIS has to be uncommonly tolerant. It appears that only quite aggressive natural infections will generate significant secretory responses. This could be considered the 'bad' side of tolerance, because it means that it is not easy to develop vaccination regimens for mucosal protection, as it is difficult to selectively induce strong responses.

Koh Fujihashi, Jerry McGhee, and co-workers have, for many years, studied the issue of mucosal vaccination in relation to both oral and intestinal infections. In their review (21) they take a closer look at the processes at work in the mucosal immune system. The group has shown that co-administration of adjuvants (molecules that help by contributing crucial signals for generation of effective responses) is necessary to obtain effective mucosal immunity through vaccination. Recently, they have studied the use, in such adjuvant roles, of mutants of cholera toxin (CT) subunit B and heat-labile toxin I from *Escherichia coli*. These toxins are potent adjuvants, but there are potential side effects with the use of some of them. For example, there may be neurotoxicity with intranasal delivery, and they can cause diarrhea. Interestingly, when the mucosal adjuvanticity of CT was evaluated in aged mice, early immune dysregulation was seen in the mucosal immune system. The last part of the review describes how

antigen feeding without adjuvant can downregulate mucosal immune responses. The group has studied the role of Peyer patches in the maintenance of such tolerance, finding that mice that lack Peyer patches retain their ability to produce secretory antibodies but do not develop oral tolerance to protein antigens. New investigations will certainly add to our knowledge of the development of oral tolerance in the near future.

*Immunological systemic and oral tolerance—the 'ugly' side*

'Ugly' features appear when tolerance fails in its mission. This happens when acquired host reactions to antigens derived from self, diet, the environment, or the normal microflora are allowed to develop. Markus Streit & Lasse Braathen (22) describe the clinical features of some allergic reactions in skin. Tony Axéll (23) does likewise for the oral mucosa. The authors point out that it is essential to distinguish between toxic (irritant) and allergic (immunological) reactions. T cells play the essential role in most allergic conditions, but antibodies may also be involved (for example, type-I hypersensitivity reaction). Metals and their compounds are important haptens in both skin and oral mucosa.

Streit & Braathen (22) describe more closely the immunological mechanisms in allergic contact dermatitis. Antigen is taken up by Langerhans cells (LC), after which the cells migrate out of the epidermis and enter the afferent lymph-vessels. On their way to the regional lymph node, the LC mature and acquire antigen-processing and -presenting capacity. In the paracortical areas of the lymph node the cells activate naive T cells, to become T effector/memory cells. The latter then enter the blood stream and, through their homing receptors, migrate through small vessels into the skin, where they can induce apoptosis of keratinocytes (10).

Tony Axèll (23) acknowledges that lichenoid reactions are the most common adverse reactions to dental materials seen in the oral cavity. The lesions have histopathological characteristics comparable to type-IV hypersensitivity reactions. Type-I reactions may also be encountered and are mostly related to the application of polymers in the oral cavity during, for example, orthodontic bonding and fissure sealing and with the use of latex gloves. Systemic symptoms such as asthmatic attacks and urticaria of the skin may accompany such reactions.

Lasse Kanerva further elaborates on the immunological adverse reactions that can be initiated by dental acrylics, which are widely used in dental filling materials and in prosthetic materials (24). Allergy to dental acrylics poses complex problems for both dental patients and personnel. A particular difficulty is that there is extensive cross-reactivity of multifunctional methacrylates and acrylates. When patients or dental personnel are sensitized with one product, it can be difficult to find other products that will be tolerated. This is further complicated by incomplete product labeling. The presence of unknown components in materials can also result in erroneous interpretation of biological tests. More precise characterization and labeling of dental acrylic materials should be given priority.

*Secreted innate immune factors are also subject to constraint*

Anatomical barriers and innate immune factors have crucial functions in protecting against infection in the respiratory, digestive, and reproductive tracts. In milk, immunoglobulins, oligosaccharides, lysozyme, lactoferrin, and complement are intended to protect the newborn. If secreted factors can induce inflammation, then the need for constraint of the reaction arises. An example of this is given in the review by Vidal and colleagues (25), who describe their discovery of a soluble form of the bacterial pattern recognition receptor CD14 in human milk. CD14 is also known as a lipopolysaccharid (LPS) receptor and is mainly found on monocytes and macrophages. These cells become activated when the CD14 receptor captures LPS that is complexed with LPS-binding protein (LBP) (3). The soluble form of CD14 also complexes with LBP/LPS and can activate cells that do not themselves carry CD14 in their cell membrane, such as epithelial cells. Vidal and colleagues (21) identified a special form of soluble CD14 in human milk (milk-soluble CD14 or m-sCD14) which mediates activation by LPS of intestinal epithelial cells. This results in release of innate immune response molecules, such as interleukin-8 and tumor necrosis factor-$\alpha$, which are important in antibacterial defense. However, the regulatory effect of LBP in this system is perhaps as important as the activation of defense systems by m-sCD14: LBP is needed in low concentrations, but when concentrations increase, it *inhibits* cytokine secretion by intestinal epithelial cells. Whether such mechanisms exist in other secretions such as saliva is an interesting issue that remains to be studied.

*Recent legislation for biological evaluation of biomaterials and medical devices in the European Union*

Evaluation and certification of biomaterials are important and demanding tasks. The European New Approach aims at ensuring free movement of goods, persons, services, and capital in the internal market (countries of the European Union (EU) and the European Economic Area Agreement (EEA)), without any restricting national regulations that may act as barriers to trade. This is regulated by European Community Directives that are transposed to national legislation in each EU/EEA country. When products are marked with the CE symbol, this signifies that the manufacturer declares that the product meets the Essential Requirements of the relevant European Directive and therefore can be freely moved within the internal market.

Dental materials fall in the category of medical devices, which are specifically regulated by the Medical Device Directive (MDD) ('Council Directive 93/42/EEC of 14 June 1993 concerning medical devices'). It has been in full effect since 14 June 1998.

Ideally, biomaterials and medical devices should perfectly fulfill the function they are designed for, without having adverse effects. Practically, however, some minor side effects can be tolerated if this is outweighed by the benefit for the patient. In Annex I, Essential Requirements, this is formally specified as follows: 'The devices must be designed and manufactured in such a way that, when used under the conditions and for the purposes intended, they will not compromise the clinical condition or the safety of the patients, or the safety and health of users, or where applicable other persons, provided that any risks which may associated with their use constitute acceptable risks when weighed against the benefits to the patient and are compatible with a high level of protection of health and safety'.

Devices must be tested for function and side effects, but it is also important that there is a quality control that ensures that they are manufactured in such a way as to guarantee the characteristics and performances referred to in the General Requirements. Particular attention must be paid to the choice of materials used and compatibility between the materials and biological tissues, cells, and body fluids, taking into account the intended purpose of the device.

Assessment of the biological properties of the individual components and the complete device may comprise literature surveys characterization, evaluation of leachable substances from the devices, biological testing under simulated laboratory conditions, and clinical testing in humans. The standardization bodies CEN and ISO have developed a series of standards for biological evaluation of medical devices for selecting relevant test methods. These tests do not cover all biocompatibility aspects and need to be used with due caution. The methods described in the standards generally do not have any pass/fail criteria. The final risk assessment needs to be performed in a structured manner by a knowledgeable person ('expert'). The final responsibility for the safety and performance of the product remains with the manufacturer.

There are severe limitations inherent in extrapolation of biological laboratory studies in relatively few animals to actual human use situations with a large number of persons. Manufacturers are required to have a structured system for postmarketing surveillance and feedback of complaints.

Many bodies (OECD, WHO, FDA, European Commission, European Parliament) are working on developing new legislation or guidelines in connection with products that may alleviate chronic disease. The immediate concerns are xenotransplants (animal tissues) and/or tissue-engineered products derived in part from the patient's own cells. Some potential problems associated with such products are related to risk of infection of humans with xenozoonoses—infections limited so far in their distribution to animal species. However, the risks associated with xenotransplantation are not only for the recipient but could be widespread in terms of public health. Future studies need to consider the human body's immunological response to antigens or infectious agents presented to 'unusual' parts of the body and how the body will cope with them.

*Internet information sources*

The 'Council Directive 93/42/EEC of 14 June 1993 Concerning Medical Devices' is available at: http://europa.eu.int/comm/dg03/directs/dg3d/d2/meddev/md/dirmd/dirmd/htm

http://europa.eu.int/comm/dgs/enterprise/index_-en.htm

http://www.eucomed.be

http://www.eotc.be/Events/index.htm

http://www.dimdi.de/engl/mpgengl/fr-euda.htm

http://www.mdc-ce.de

Information about the new approach to technical harmonization and standardization, Harmonized Standards, can be found at http://europa.eu.int/comm/enterprise/newapproach/standardization/index.html.

References to harmonized standards under the 'New Approach Directives' is on http://europa.eu.int/comm/enterprise/newapproach/standardization/harmstds/index.html

For an overview of all healthcare sector standards, see: http://www.cenorm.be/sectors/healthcare.htm

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